

Fig 1. Diagrammatic Overview of the invention

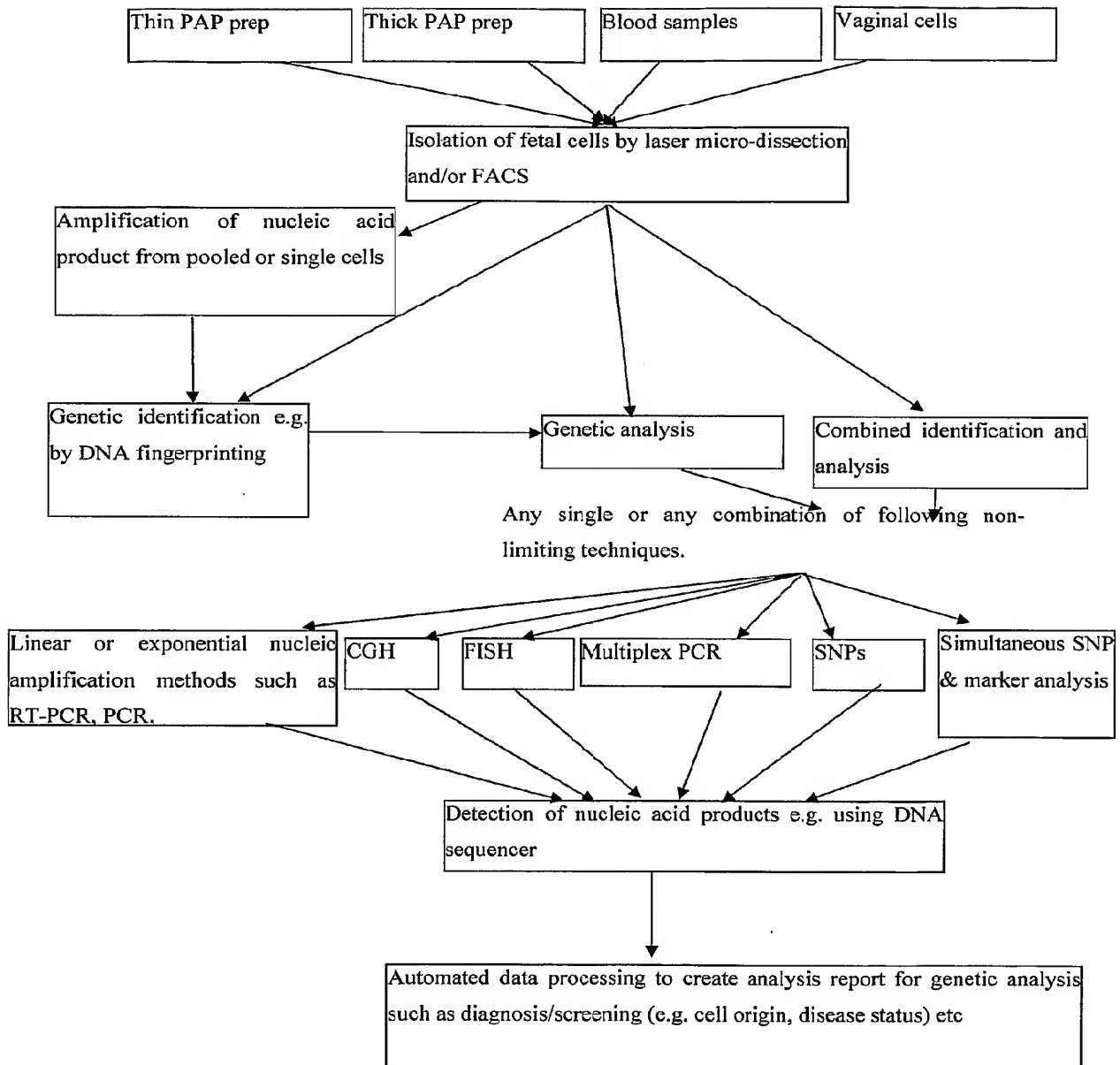


Fig 2 (TABLE 1 – STR markers used for DNA identification and genetic analysis)

MARKER	ALIAS	GENBANK		PRIMER SEQUENCE
		NO.	POSITION	
D13S241	UT556	L17673	13pter	CCA GGC ACT TTG GGA GGC TG ACC CAC TGT ATC CTG GGC A
D13S242	UT557	L18329	13q21.2	ATT GCA CCC CAT CCT GGG TCC TTT TCC TAC CAT TTG CAT
D13S243	UT558	L18330	13cen-13q12.1	ACT GTA CTT CTG CCT GGG C TTT TGT AAT GCC TCA ACC ATG
D13S248	UT1213	L15541	13q32-13q34	ACT TAA ATG TCC ATC AAT AAA T TGA TTG GCT TTT TTT ACT TAC
D13S251	UT1329	L16338	13q31-13q32	CAC ATA GCT TAT TGT TGT TGC GTT ATC TGT GAG CAA ATA CAG
D13S253	UT1378	L16396	13q22-13q32	CTC AAG GGA TGT TAA CAC AC AGG AGG AAA AAG TGG AGA AG
D13S254	UT1585	L18690	13q31-13q32	TGA ACT CCG GCC TGG GTG A TTT TGG AGC TGG GGA TGT C
D13S256	UT2120	L17977	13q14.1-13q22	CCT GGG CAA CAA GAG CAA A AGC AGA GAG ACA TAA TTG TG
D13S257	UT2119	L18729	13q14.1-13q21.1	CAA CAA GAG CAA AAC TCC AT AAG CAC ATA AGT TGG TAT GAA
D13S258	UT2413	L18095	13q21.2-13q31	ACC TGC CAA ATT TTA CCA GG GAC AGA GAG AGG GAA TAA ACC
D13S303	UT936	L31309	13q22-13q31	ACA TCG CTC CTT ACC CCA TC TGT ACC CAT TAA CCA TCC CCA
D13S631	UT7403	L18392	13q31-13q32	GGC AAC AAG AGC AAA ACT CT TAG CCC TCA CCA TGA TTG G
D18S51	UT574	L18333	18q21.33- 18q21.33	GAG CCA TGT TCA TGC CAC TG CAA ACC CGA CTA CCA GCA AC

D18S378	UT485	L16262	18p11.22- 18p11.22	AGC CTG GGT GAC AGA GCA A ACA GGG AAA GCT GGG GGA T CAT CCA TCC ATC CTT CCA C TGT GCT GGT ATT ACA GGC G TCA GGA GAA TCA CTT GGA AC TCC ATG AAG TAG CTA AGC AG TAA CCA AAG CAA ATC CCT GG CAC TTA CAC TGT TAT CCT GG CTG GTT TTC GTC TTG AGA AG CAC TAT TCC CAT CTG AGT CA CTT CCC TGG GTA TCA AGA CT TCC CAC TAT ATG TAT GTT CAC C GGC TGA GAC AGG AGA ATC AC CTC ACC AGG ATT TCC TTG C ACC ACA GTT ACT AAG ATG TAA GCC TCC AGA AAA AAT TTC CA CTG TCC TCT AGG CTC ATT TAG C TTA TGA AGC AGT GAT GCC AA GTG AGT CAA TTC CCC AAG GTT GTA TTA GTC AAT GTT CTC C GAG ACG GTA GGA AAA GGA G AGC CAA GTT CGA GCC ACT G GTC CCC ATA TTG ATA AAC TAT T ATG AAT AGG GGA TAT GCT GG TTG CAG GGA AAC CAC AGT T TCC TTG GAA TAA ATT CCC GG CGG AGG TTG CAG TGA GTT G GGG AAG GCT ATG GAG GAG A ATG ATG AAT GCA TAG ATG GAT G AAT GTG TGT CCT TCC AGG C
D18S382	UT600	L16292	18pter-18pter	
D18S386	UT754	L18400	18q22.1-18q22.2	
D18S390	UT1227	L15542	18q22.3-18q23	
D18S391	UT1302	L16384	18pter-18p11.22	
D18S814	UT1248	L17776	18pter-18pter	
D18S815	UT1438	L17819	18pter-18qter	
D18S819	UT7251	L30411	18pter-18qter	
D18S851	SHGC 4561	G08002	18pter-18qter	
D21S11	VS1773	M84567	21q21-21q21	
D21S1240	UT656	L18360	21pter-21qter	
D21S1244	UT761	L16331	21q21-21q22.1	
D21S1413	UT7582	L30513	21pter-21pter	
D21S1412	UT6930	L29680	21pter-21pter	
D21S1411	UT1355	L17803	21pter-21pter	

PENTA E	PAUL1	AC027004 21q	CCC TGG GCT CTG TAA AGA ATA GTG
AMEL		M55418	ATC AGA GCT TAA ACT GGG AAG CTG
HUMTHO		D00269 11p15-15.5	GCT TCC GAG TGC AGG TCA CA
			CAG CTG CCC TAG TCA GCA C
TPOX		M68651 2p23-2pter	CAC TAG CAC CCA GAA CCG TC
			CCT TGT CAG CGT TTA TTT GCC
VWA		M25858 12p12-pter	CCC TAG TGG ATG ATA AGA ATA ATC AGT ATG
			GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG
D3S1358		11449919 3p	ACT GCA GTC CAA TCT GGG T
			ATG AAA TCA ACA GAG GCT TG
D5S818		G08446 5q21-q31	GGG TGA TTT TCC TCT TTG GT
			TGA TTC CAA TCA TAG CCA CA
D7S820		G08616 7q	TGT CAT AGT TTA GAA CGA ACT AAC G
			CTG AGG TAT CAA AAA CTC AGA GG
CSF1PO	U63963	X14720 5q33.3-34	AAC CTG AGT CTG CCA AGG ACT AGC
			TTC CAC ACA CCA CTG GCC ATC TTC
FGA		M64982 4q28	GCC CCA TAG GTT TTG AAC TCA
			TGA TTT GTC TGT AAT TGC CAG C
D13S317		G09017 13q22-q31	ACA GAA GTC TGG GAT GTG GA
			GCC CAA AAA GAC AGA CAG AA
DYS14			CTT TCC ACA GCC ACA TTT GTC
			X CAT CCA GAG CGT CCC TGG CTT
D13S622		990	
D13S304		937	
D13S247		991	
D13S621		642	
D13S250		1250	
D13S633		7708	
D13S243		558	

D13S625	1587
D13S246	740
D13S252	1352
D13S629	6870
D13S624	5236
D13S305	5177
D13S240	472
D13S249	1222
D13S257	2119
D13S256	2120
D13S626	5570
D13S242	557
D13S634	7875
D13S258	2413
D13S303	936
D13S921	2347
D13S251	1329
D13S628	6073
D13S253	1378
D13S627	5821
D13S631	7403
D13S254	1585
D13S248	1213
D18S999	7873
D18S820	7913
D18S818	7162
D18S391	1302
D18S378	485
D18S819	7251
D18S816	5780

CCA GGC ACT TTG GGA GGC TG
ACC CAC TGT ATC CTG GGC A
TGG AAG GTC GAA GCT GAA GTG A
CCT GTG GCG TGT CTT TTT ACT TTC T
ATC ACT TGA ACC CAG GAG GTG GA

D8S1179e	G08710	8q24	GGG GAG GCT GTG TAA GAA GTG TT TTT GGC CAG AAA CCT CTG TAG CC
CSF1POe	X14720	5q33	AAC TGA AAC CCT GTG CAT TGT TGT TG TCC AAC CTG AGT CTG CCA AGG A
D5S818e	G08446	5q21	CTT CCA CAC ACC ACT GGC CAT CTT ACA AGG GTG ATT TTC CTC TTT GGT ATC
D7S820e	G08616	7q	CCA AGT GAT TCC AAT CAT AGC CAC A TGT CAT AGT TTA GAA CGA ACT AAC GAT AG
D21S11e	M84567	21p11.1	AAA TCT GAG GTA TCA AAA ACT CAG AGG AAT ATG TGA GTC AAT TCC CCA AGT GAA T
FGAe	M64982	4q28	TGT ATT AGT CAA TGT TCT CCA GAG ACA TGC CCC ATA GGT TTT GAA CTC ACA
TPOXe	M68651	2p23	GAG TGA TTT GTC TGT AAT TGC CAG C CAC TAG CAC CCA GAA CCG TCG
THOe	D00269	11p15	TGT CCT TGT CAG CGT TTA TTT GCC GTG GGC TGA AAA GCT CCC GAT
D18S51e	AP001534	18q21.3	GTG ATT CCC ATT GGC CTG TTC CTC TGA GCC ATG TTC ATG CCA CTG
D3S1358e	11449919	3p21	ACA AAC CCG ACT ACC AGC AAC TT ACT GCA GTC CAA TCT GGG TGA CAG
BKMDY1		Y	ATG AAA TCA ACA GAG GCT TGC ATG TAT C GAA GTG CTC GGC ATT GTT AGG AT
BKMDY2		Y	AGA TCC ATT TGC AGA CTG CCT TAT AAG TGC TCG GCA TTG TTA GGA TT
D13S317e	G09017	13q22-q31	CTA AGC AGA TCC ATT TGC AGA CT CTT CCT ACC ACT GAA CAT AAA CTG CTT AA
D13S258e	L18095	13q21.2-13q31	CAG TGA GCC AAG GTC GTG CCA ACC TGC CAA ATT TTA CCA GGA GGA GAC AGA GAG AGG GAA TAA ACC AAT AAG A

D13S631e	UT7403	L18392	13q31-13q32	GGC AAC AAG AGC AAA ACT CTG C TGG AAA AAT AAT TTC TGG GGG TGG GA CTG GTT TTC GTC TTG AGA AGT CAT G CAC TAT TCC CAT CTG AGT CAC TCA G ACA CAC ACA AAC ATC TCT TTC TAT CTA TAT A GCC TTT ATG AAG CAG TGA TGC CAA ATG ATG AAT GCA TAG ATG GAT GGA TG AAT GTG TGT CCT TCC AGG CTT TCT CGG AGG TTG CAG TGA GTT GAG GGG AAG GCT ATG GAG GAG A TTG CAG GGA AAC CAC AGT TAT ACA TTC TCC TTG GAA TAA ATT CCC GGA AGT TTT CAT CCA GAG CGT CCC TGG C GCT TTC CAC AGC CAC ATT GGT CC CCA GGC ACT TTG GGA GGC TG ACC CAC TGT ATC CTG GGC A TGG AAG GTC GAA GCT GAA GTG A CCT GTG GCG TGT CTT TTT ACT TTC T ATC ACT TGA ACC CAG GAG GTG GA GGG GAG GCT GTG TAA GAA GTG TT TTT GGC CAG AAA CCT CTG TAG CC AAC TGA AAC CCT GTG CAT TGT TGT TG TCC AAC CTG AGT CTG CCA AGG A CTT CCA CAC ACC ACT GGC CAT CTT ACA AGG GTG ATT TTC CTC TTT GGT ATC CCA AGT GAT TCC AAT CAT AGC CAC A TGT CAT AGT TTA GAA CGA ACT AAC GAT AG AAA TCT GAG GTA TCA AAA ACT CAG AGG AAT ATG TGA GTC AAT TCC CCA AGT GAA T
D18S391e	UT1302	L16384	18pter-18p11.22	
D18S851e	SHGC 4561	G08002	18pter-18qter	
D21S1411e	UT1355	L17803	21pter-21pter	
D21S1412e	UT6930	L29680	21pter-21pter	
D21S1413e	UT7582	L30513	21pter-21pter	
DYS14e	See TSPY			
D13S241	UT556	L17673	13pter	
PD1		AP001752	21q	
PE1		AC027004	15q	
D8S1179e		G08710	8q24	
CSF1POe		X14720	5q33	
D5S818e		G08446	5q21	
D7S820e		G08616	7q	
D21S11e		M84567	21p11.1	

FGAe	M64982	4q28	TGT ATT AGT CAA TGT TCT CCA GAG ACA TGC CCC ATA GGT TTT GAA CTC ACA GAG TGA TTT GTC TGT AAT TGC CAG C CAC TAG CAC CCA GAA CCG TCG TGT CCT TGT CAG CGT TTA TTT GCC GTG GGC TGA AAA GCT CCC GAT GTG ATT CCC ATT GGC CTG TTC CTC TGA GCC ATG TTC ATG CCA CTG ACA AAC CCG ACT ACC AGC AAC TT ACT GCA GTC CAA TCT GGG TGA CAG ATG AAA TCA ACA GAG GCT TGC ATG TAT C GAA GTG CTC GGC ATT GTT AGG AT AGA TCC ATT TGC AGA CTG CCT TAT AAG TGC TCG GCA TTG TTA GGA TT CTA AGC AGA TCC ATT TGC AGA CT CTT CCT ACC ACT GAA CAT AAA CTG CTT AA CAG TGA GCC AAG GTC GTG CCA ACC TGC CAA ATT TTA CCA GGA GGA GAC AGA GAG AGG GAA TAA ACC AAT AAG A GGC AAC AAG AGC AAA ACT CTG C TGG AAA AAT AAT TTC TGG GGG TGG GA CTG GTT TTC GTC TTG AGA AGT CAT G CAC TAT TCC CAT CTG AGT CAC TCA G ACA CAC ACA AAC ATC TCT TTC TAT CTA TAT A GCC TTT ATG AAG CAG TGA TGC CAA ATG ATG AAT GCA TAG ATG GAT GGA TG AAT GTG TGT CCT TCC AGG CTT TCT CGG AGG TTG CAG TGA GTT GAG GGG AAG GCT ATG GAG GAG A
TPOXe	M68651	2p23	
THOe	D00269	11p15	
D18S51e	AP001534	18q21.3	
D3S1358e	11449919	3p21	
BKMDY1		Y	
BKMDY2		Y	
D13S317e	G09017	13q22-q31	
D13S258e	L18095	13q21.2-13q31	
D13S631e	L18392	13q31-13q32	
D18S391e	L16384	18pter-18p11.22	
D18S851e	G08002	18pter-18qter	
D21S1411e	L17803	21pter-21pter	
D21S1412e	L29680	21pter-21pter	

D21S1413e	UT7582	L30513	21pter-21pter	TTG CAG GGA AAC CAC AGT TAT ACA TTC
DYS14e	See TSPY			TCC TTG GAA TAA ATT CCC GGA AGT TTT
				CAT CCA GAG CGT CCC TGG C
				GCT TTC CAC AGC CAC ATT GGT CC

Fig 3 (TABLE 2 Examples of Markers used for genetic analysis embodiment)

Primer set	Fluorescent Dye	pmoles
Amelogenin	FAM	Variable from 1-40
DYS14	FAM	Variable from 1-40
D21S11	FAM or TET	Variable from 1-40
D13S631	HEX	Variable from 1-40
D13S258	HEX	Variable from 1-40
D18S51	FAM	Variable from 1-40
D18S851	FAM	Variable from 1-40
D18S391	HEX	Variable from 1-40
D13S317	TET	Variable from 1-40
D21S1413	HEX	Variable from 1-40
D21S1412	TET	Variable from 1-40
D21S1411	FAM	Variable from 1-40

Fig 4 (TABLE 3 Example of Markers used for DNA identification embodiment)

<u>Primer set</u>	<u>Fluorescent Dye</u>	<u>pmoles</u>
Amelogenin	FAM	Variable from 1-40
HUMTHO	FAM	Variable from 1-40
D21S11	FAM	Variable from 1-40
D18S51	FAM	Variable from 1-40
VWA	HEX	Variable from 1-40
FGA	HEX	Variable from 1-40
D3S1358	FAM	Variable from 1-40
D5S818	TET	Variable from 1-40
D7S820	TET	Variable from 1-40
CSF1PO	HEX	Variable from 1-40
TPOX	TET	Variable from 1-40

Fig 5 (TABLE 4 Comparison of the efficacy of various analytical methods)

	Fluorescent PCR	FISH	Conventional PCR	PRINS
High reliability	97%	86%	84% for CF	91%
High accuracy	97-99% 97% for (Carrier) in CF	>95%	79% (Unaffected) & 66% (Carrier) for CF	25%
Rapid diagnosis	6 hrs	~4 hrs	8-10 hrs	6 hrs
Diagnosis of sex	Yes	Yes	Possible but poor reliability & accuracy	Yes
Diagnosis of single-gene defects	Yes	No	Yes	No
Diagnosis of trisomies	Yes	Yes	No	Yes
Confirmation of diagnosis	Yes	No	No	No
DNA fingerprinting	Yes, high specificity 1 in billions.	No	Limited ~1 in 10	No
Detection of contamination	Yes	No	Very limited	No
Simultaneous diagnoses	Sex, CF, trisomies & DNA fingerprint	Trisomies	No	No
No of chromosomes simultaneously analysed	Potentially all chromosomes	3-5	1	3

Fig 6 Dot plots generated by the Beckman Coulter EXPO32 analysis software showing the effects of cell solution filtration on cell sorting. Column 1 shows a cell population screened with a 75µm filter, eliminating all large particles but leaving debris (grey). Column 2 shows a cell population filtered at 75µm, and once at 15µm. Column 3 shows a cell population first filtered at 75µm, then twice at 15µm. The cells of interest are coloured green whilst debris is coloured grey. The proportion of the green coloured target cells can be seen to increase with filtration whilst the proportion of debris decreases. Relative percentages are noted on the lower graphs

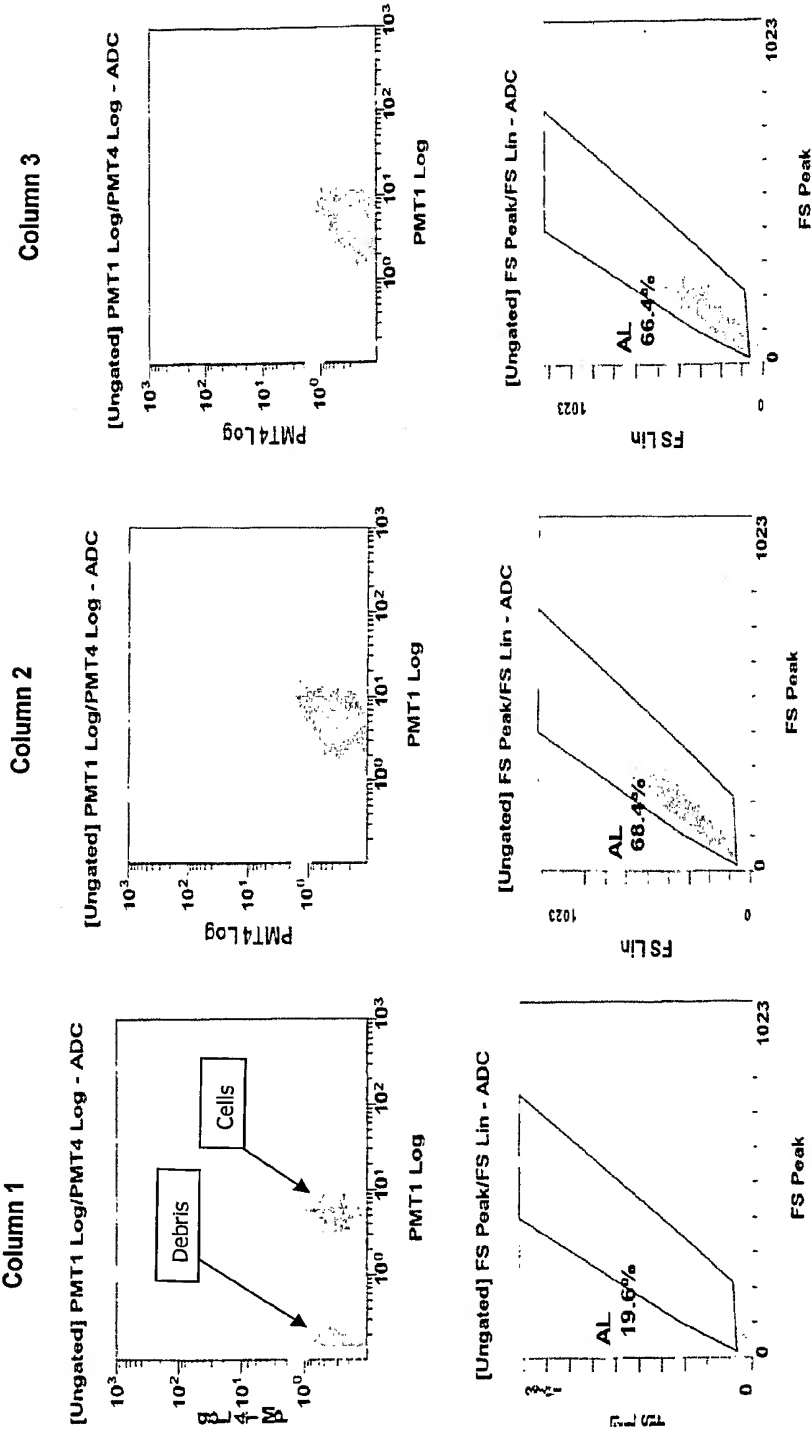
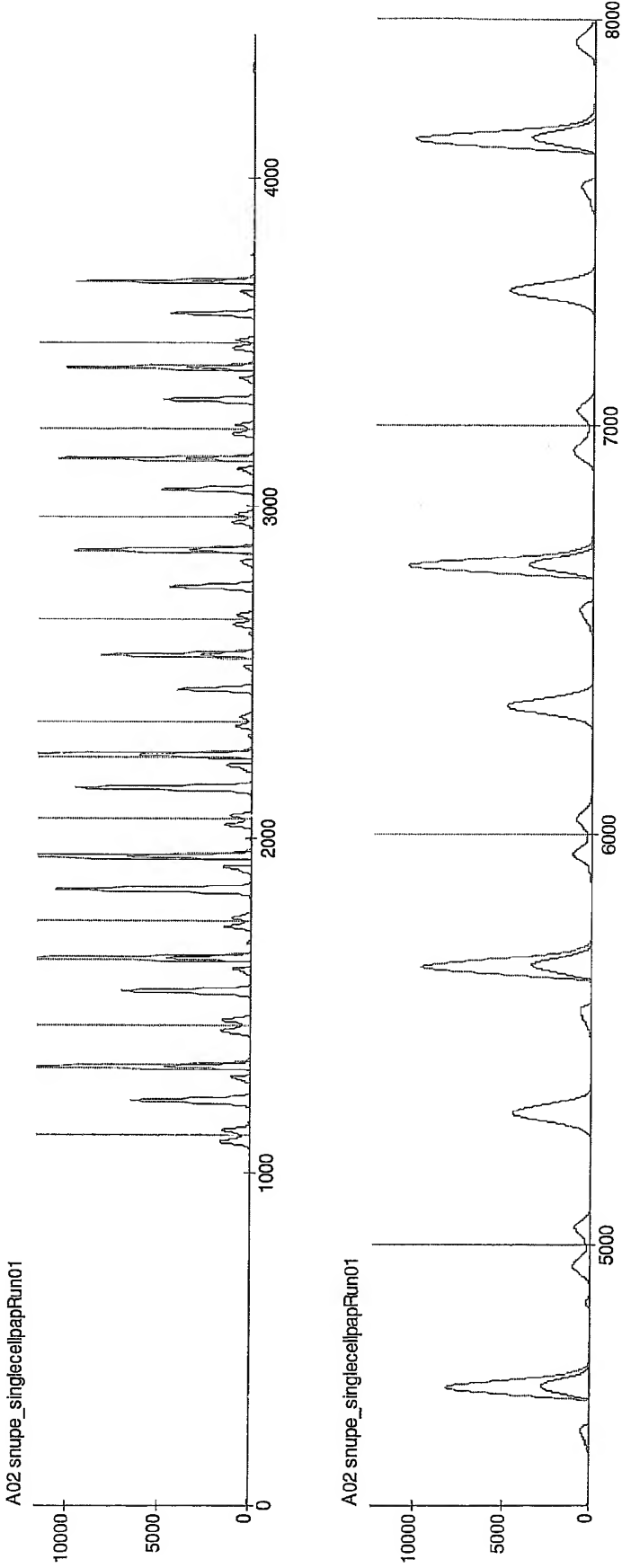


Fig 7 Single cell SNP results

Duplex SNP Reaction

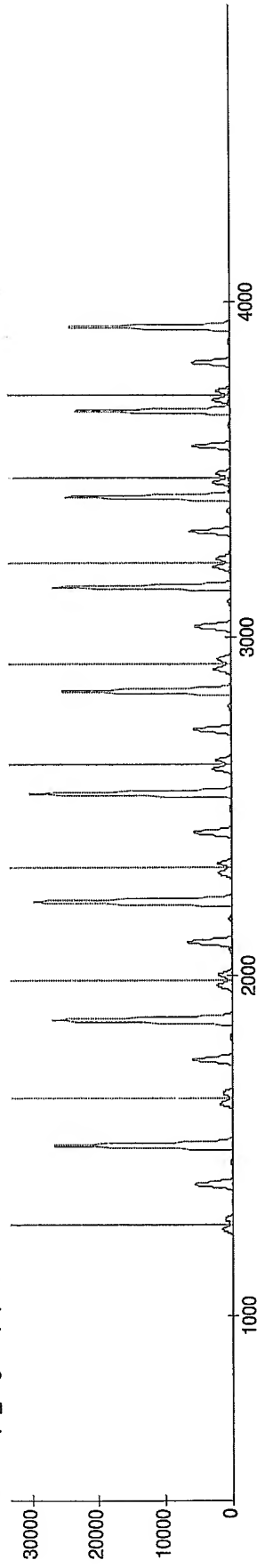
A reaction was performed combining two SNPs KEL and RhE in a single reaction. Run in 80 single cells, 25% produced a clean, easily analysable result. A further 15-20% produced results which indicated that further optimisation was required for automated analysis . A further 10% produced a result for a single SNP only.

Duplex Sample: showing a C homozygote call for RhE and a CT heterozygote call for KEL.



Duplex Sample: showing a C homozygote call for RhE and a T homozygote call for KEL.

A04 snupe_singlecellpapRun01



A04 snupe_singlecellpapRun01

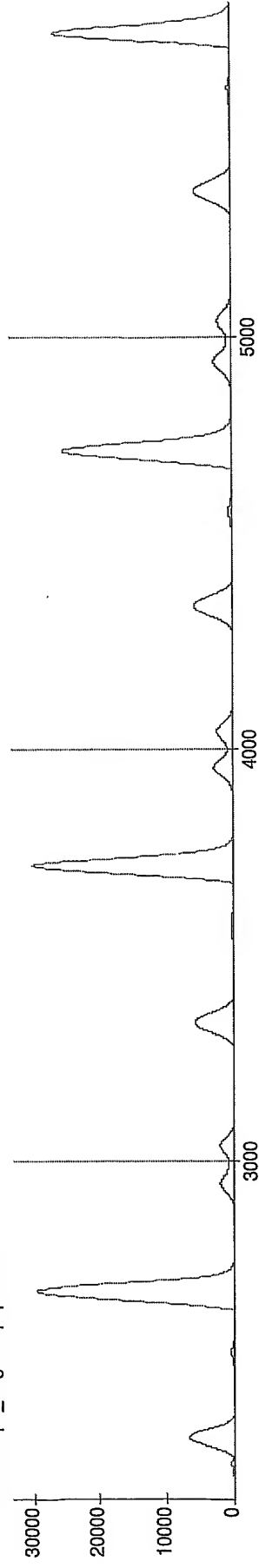
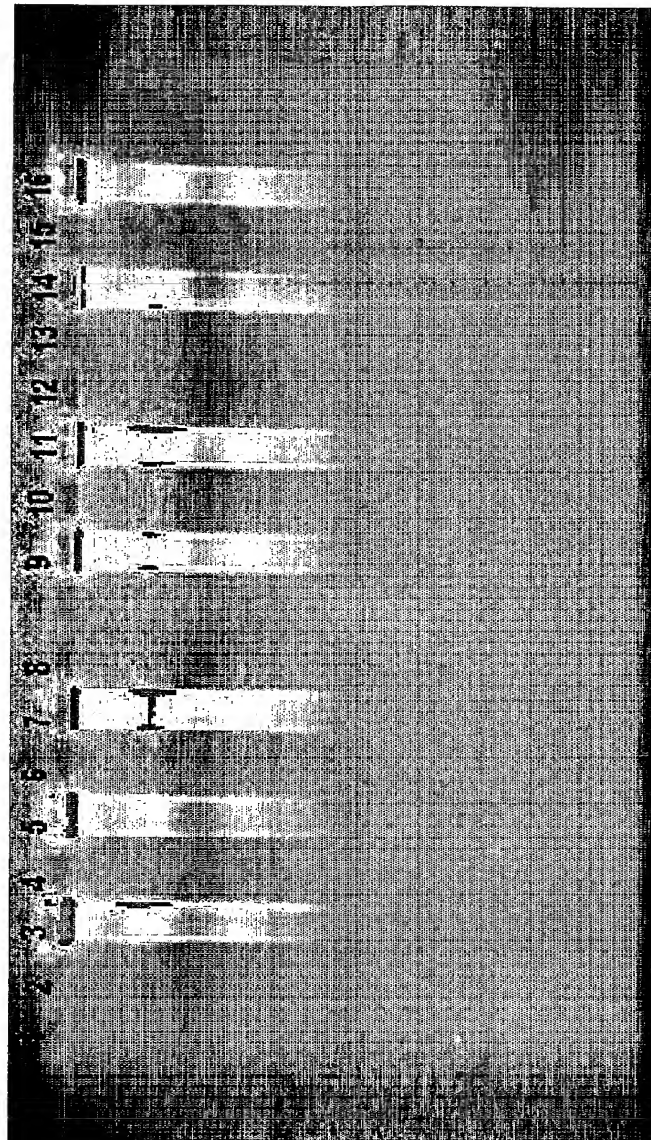
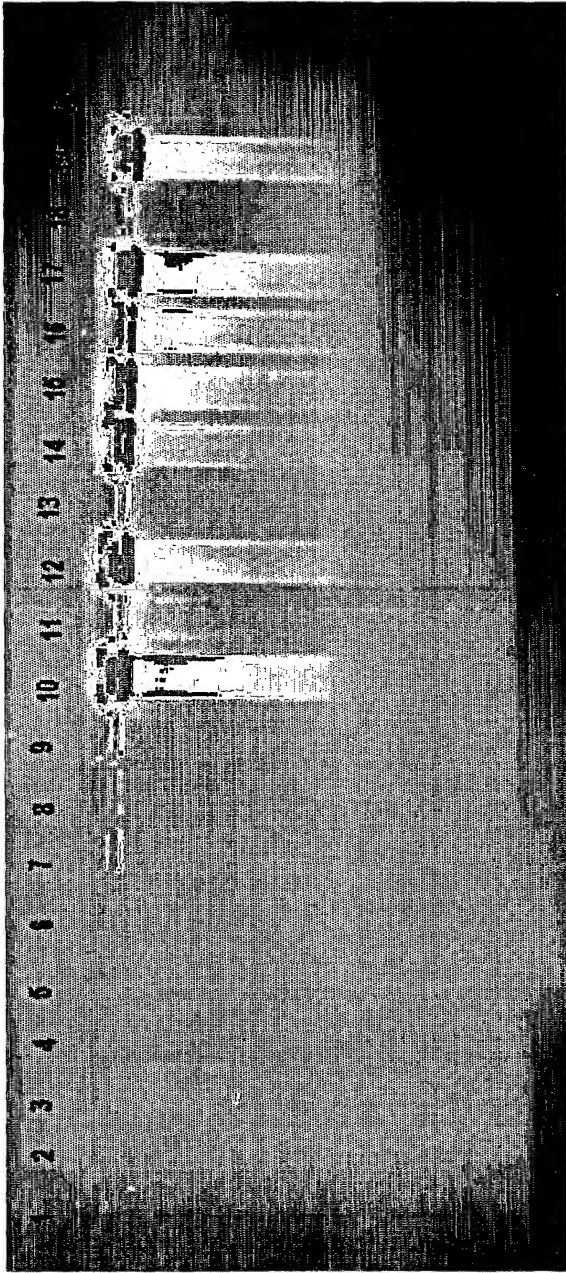


Fig 8 Single Cell Genomiphi results

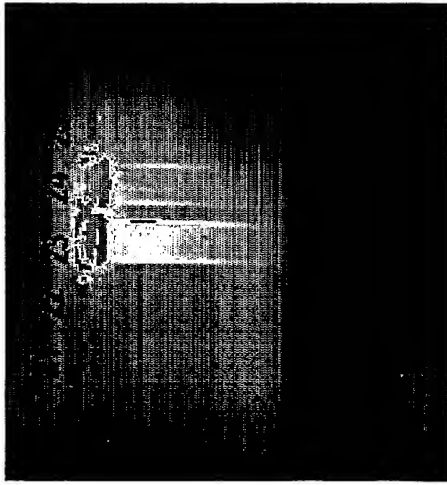


1 - 16 Single cells, picked and lysed, before being subjected to the Genomiphi reaction. These typical results indicate that current genomiphi protocols are successful in only ~44% (7/16) of samples analysed,



Genomiphi incubation including Betaine

- 1 - 5 Single cells picked from Pap smear sample and lysed with no Betaine
- 6-10 Single cells picked from Pap smear sample and lysed with 0.5M Betaine
- 11-15 Single cells picked from Pap smear sample and lysed with 1M Betaine
- 16-20 Single cells picked from Pap smear sample and lysed with 0.25M Betaine



21-25 Single cells picked from Pap smear sample, lysed and incubated with 0.75M Betaine

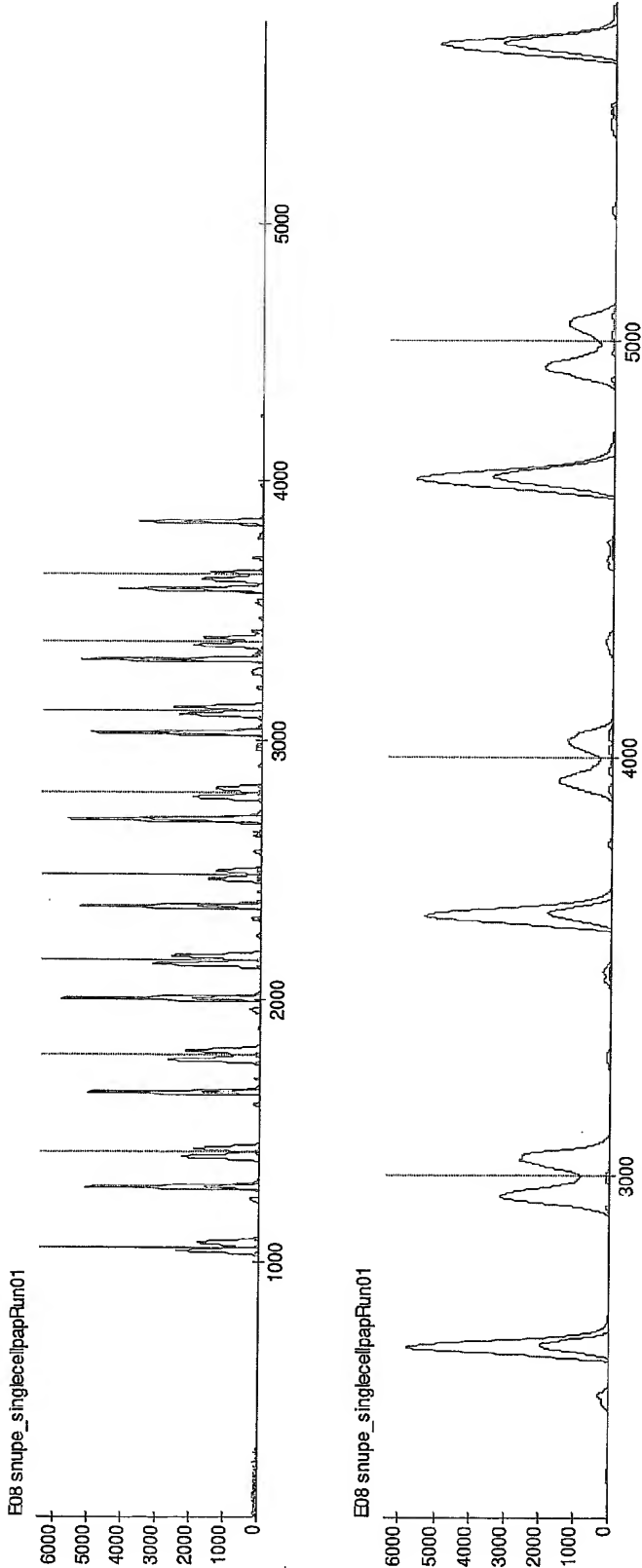
The results, using the Betaine modified protocols, indicates that not only does reliability increases with betaine use but amount of DNA yield per single cell also increased.

Single cells processed using these modifications also have reduced rates of allelic dropout, whole locus dropout and preferential amplification.

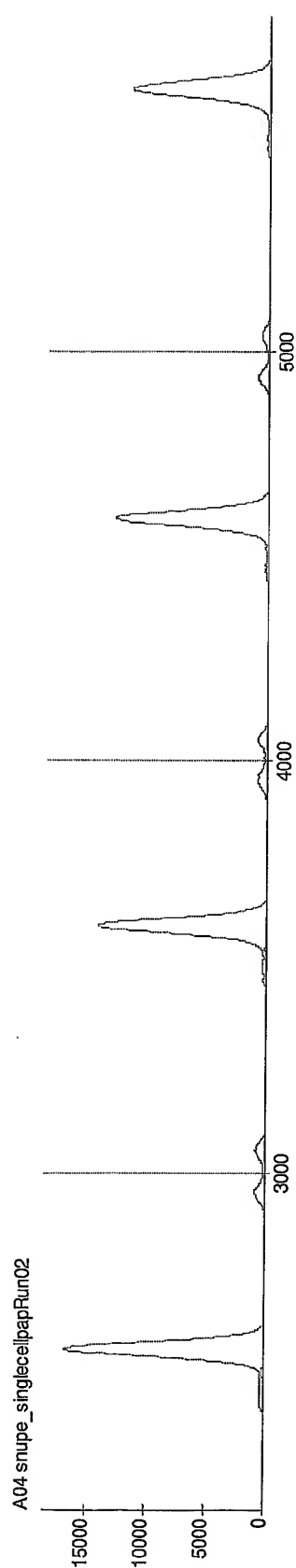
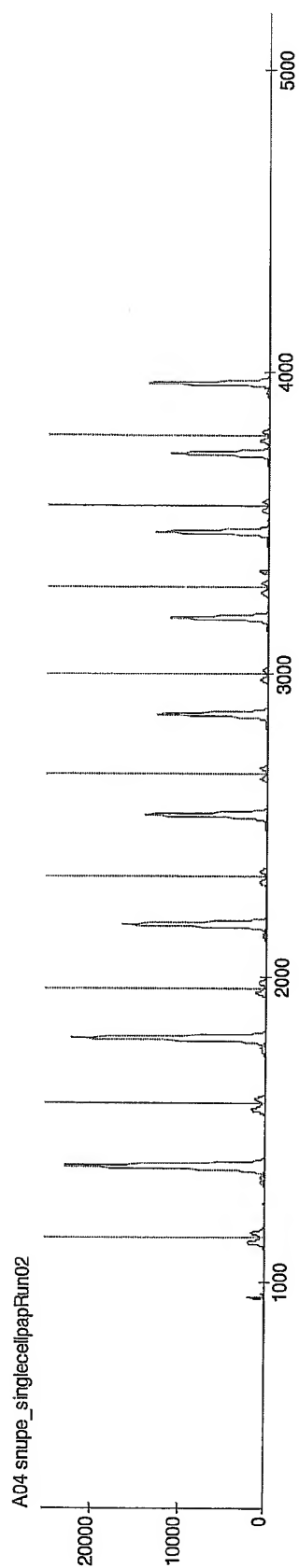
Fig 9 Uniplex SNP reaction

A similar plate of 80 samples was run using single plex reactions. The plate was split, half for RhE and half for KEL analysis. Of the RhE plate ~60% produced results. Of the KEL plate ~75% produced a result. The remaining percentage either failed or were unreadable. As with all single cell analysis systems, a significant reason for failure is that the single cell may have become lost during the process resulting in amplification failure.

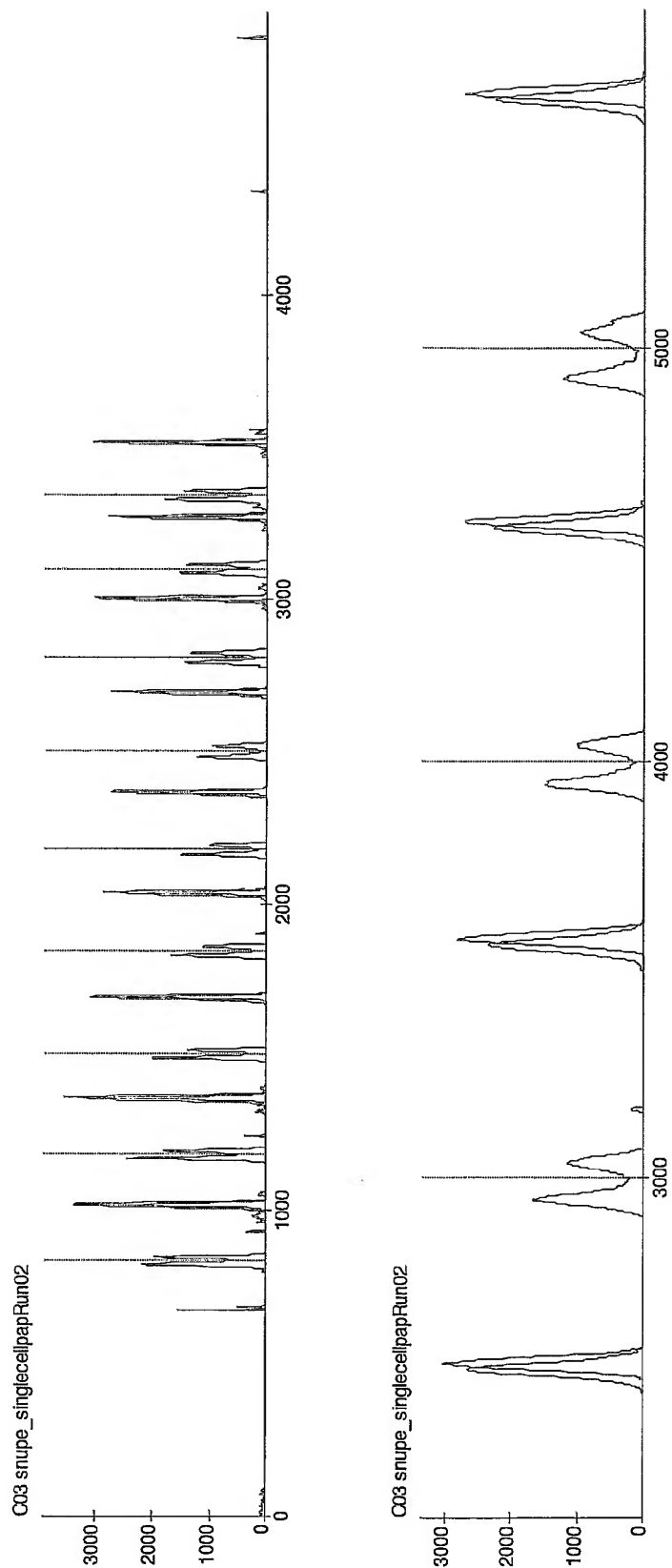
Uniplex Sample: showing a GC heterozygote call for RhE.



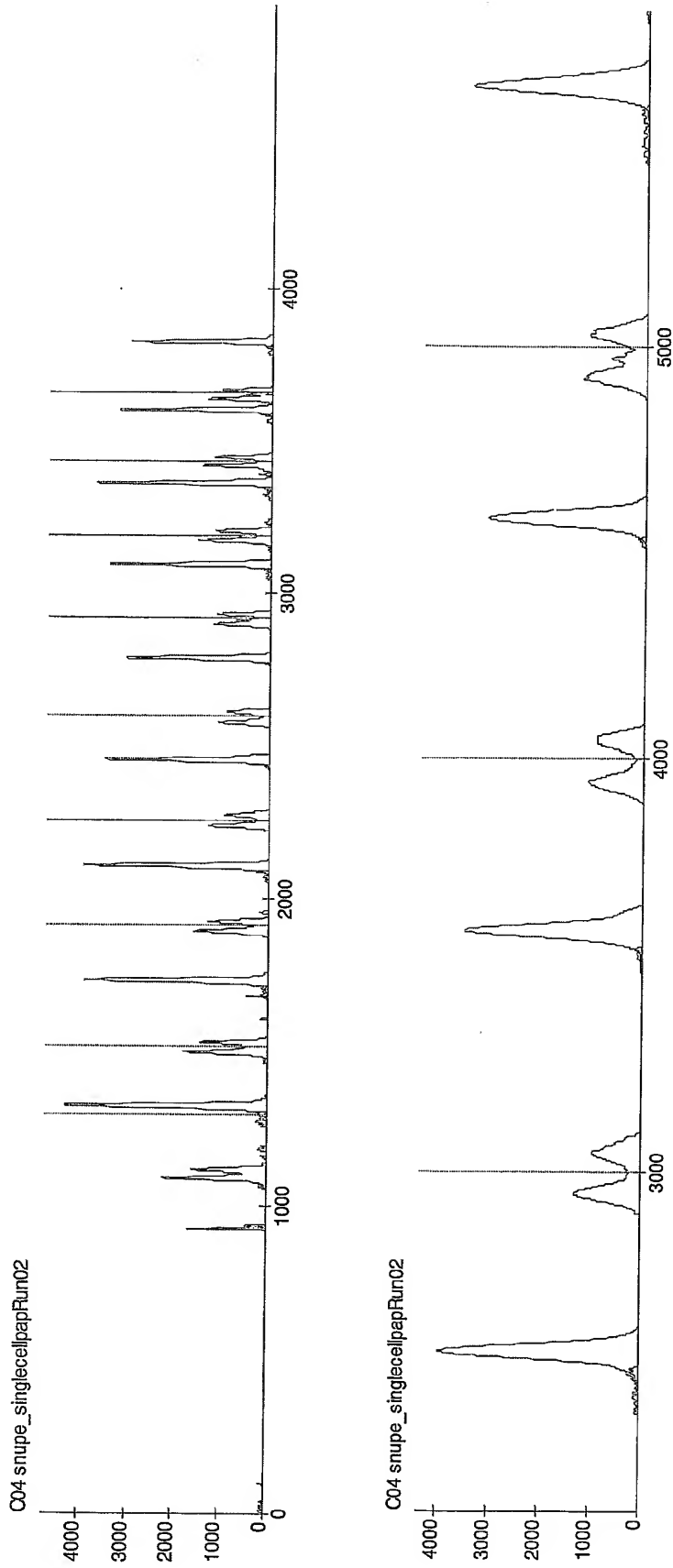
Uniplex Sample: showing a T homozygote call for KEL



Uniplex Sample: showing a GC heterozygote call for RhE.



Uniplex Sample: showing a C homozygote call for KEL.



Uniplex Sample: showing a CT heterozygote call for KEL.

